

B7  
sequence, GAAAAGCGAA (SEQ ID NO:10), is found at positions -846 to -837, i.e., within the area which the deletion results indicate controls suspensor-specific activity. Identical motifs can also be found at positions -1144 through -1135 and between -713 through -704 of Figure 1. The motif is also found at positions -684 through -675 of the Scarlet Runner Bean C541 promoter region (Figure 4). Interestingly, the Arabidopsis G564 ortholog promoter region comprises a motif (GAAAAGCCAA - SEQ ID NO:12) that is highly homologous to SEQ ID NO:10.

---

✓  
Please cancel the present informal "SEQUENCE LISTING", pages 70-80, and insert therefor the accompanying paper copy of the Sequence Listing, page numbers 1 to 23, at the end of the application. Cancel the page numbers for the Claims and Abstract and renumber, accordingly.

#### REMARKS

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-38, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification by the current Amendment. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

WETERINGS *et al.*  
Application No.: 09/724,857  
Page 7

PATENT

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Matthew E. Hinsch', is written over a horizontal line.

Matthew E. Hinsch  
Reg. No. 47,651

TOWNSEND and TOWNSEND and CREW LLP  
Two Embarcadero Center, 8<sup>th</sup> Floor  
San Francisco, California 94111-3834  
Tel: (415) 576-0200  
Fax: (415) 576-0300  
MEH:dmw

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Specification:**

Paragraph beginning at line 6 of page 17 has been amended as follows:

Figure 2 displays the nucleotide sequence (SEQ ID NO:2) of genomic DNA comprising the G564 coding sequence (amino acid sequence = SEQ ID NO:3) and promoter region from Scarlet Runner Bean (*Phaseolus coccineus*). The ATG start codon is displayed in bold and underlined nucleotides indicates intron sequences.

Paragraph beginning at line 10 of page 17 has been amended as follows:

Figure 3 displays the nucleotide sequence (SEQ ID NO:4) of genomic DNA comprising the G564 coding sequence (amino acid sequence = SEQ ID NO:5) and promoter region from *Arabidopsis thaliana*. The ATG start codon is displayed in bold and underlined nucleotides indicates intron sequences.

Paragraph beginning at line 13 of page 17 has been amended as follows:

Figure 4 displays the nucleotide sequence (SEQ ID NO:6) of genomic DNA comprising the C541 coding sequence (amino acid sequence = SEQ ID NO:7) and promoter region from Scarlet Runner Bean (*Phaseolus coccineus*). The ATG start codon is displayed in bold and underlined nucleotides indicates intron sequences.

Paragraph beginning at line 17 of page 17 has been amended as follows:

Figure 5 displays the nucleotide sequence (SEQ ID NO:8) of genomic DNA comprising the C541 coding sequence (amino acid sequence = SEQ ID NO:9) and promoter region from *Arabidopsis thaliana*. The ATG start codon is displayed in bold and underlined nucleotides indicates intron sequences.

Paragraph beginning at line 28 of page 17 has been amended as follows:

Figure 8 identifies a number of promoter control elements (SEQ ID NOS:15-24) found within sequences -921 to -662 of Figure 2 (SEQ ID NOS:13 and 14) ~~Figure 1.~~

Paragraph beginning at line 13 of page 52 has been amended as follows:

A cDNA library of 5-9 DAP Scarlet Runner Bean seeds containing globular-stage embryos was constructed using the ZAP Express® cDNA synthesis kit (Stratagene: La Jolla, CA). Poly(A) mRNA was used as a template to generate first-strand cDNA using MMLV reverse transcriptase and a 50-base oligonucleotide linker-primer [5'-(GA)<sub>10</sub>ACTAGTCTCGAG(T)<sub>18</sub>-3' (SEQ ID NO:25)]. Double-strand cDNAs were blunt-ended and ligated to an EcoRI adapter. After phosphorylation of EcoRI 5' ends, the cDNAs were digested with XhoI and size-fractionated on a Sephacryl S-400 column to exclude cDNAs that were smaller than 250 bp. The fractionated cDNAs were ligated to the λZAP vector. About 3,000 recombinants from the unamplified library were differentially screened with <sup>32</sup>P-labeled first-strand cDNAs generated from: (1) 5-9 DAP seed micropylar region poly(A) mRNA and (2) leaf poly(A) mRNA. cDNA clones representing mRNAs preferentially present in the micropylar region were screened two more times following the strategy used in the primary screen.

Paragraph beginning at line 27 of page 52 has been amended as follows:

Differential display procedures of Liang and Pardee (Liang, P., *et al.*, *Science*, 257:967-971 (1992)) were followed using the RNAimage™ kit (GenHunter Corp.: Nashville, TN). Differential display reactions were carried out using total RNA templates from: (1) 6-8 DAP dissected suspensors of globular-stage embryos, (2) 6 DAY embryo-containing micropylar seed regions, (3) 6 DAP non-embryo-containing chalazal seed regions, (4) 6-8 DAP isolated globular-stage embryo proper, (5) leaves, (6) ovules, (7) 2 DAY whole seeds, and (8) 3 DAP whole seeds. Briefly, first-strand cDNAs were generated by reverse transcription (RT) of 200 ng of total RNA using MMLV reverse transcriptase and an anchor/reverse primer (G primer: 5'-AAGCT<sub>11</sub>G-3' (SEQ ID NO:26) or C primer: 5'-AAGCT<sub>11</sub>C-3' (SEQ ID NO:27)). Aliquots of the first-strand cDNAs were used as templates for the polymerase chain reaction (PCR) using combinations of forward and anchor/reverse primers in the presence of <sup>33</sup>P-dCTP and AmpliTaq® polymerase (Perkin Elmer; Branchburg, NJ). The forward primers used were: H-AP49, 5'-AAGCTTTAGTCCA-3' (SEQ ID NO:28); H-AP50, 5'-AAGCTTTGAGACT-3' (SEQ ID NO:29); H-AP51, 5'-AAGCTTCGAAATG-3' (SEQ ID NO:30); H-AP52, 5'-AAGCTTGACCTTT-3' (SEQ ID NO:31); H-AP53, 5'-AAGCTTCCTCTAT-3' (SEQ ID NO:32); H-AP54, 5'-AAGCTTTTGAGGT-3' (SEQ ID NO:33); H-AP55, 5'-AAGCTTACGTTAG-3' (SEQ ID NO:34); and H-AP56, 5'-AAGCTTATGAAGG-3' (SEQ ID NO:35), where H-AP refers to the primers supplied by the RNAimage™ kit. The RT-PCR products were size-fractionated in a 6% acrylamide gel and visualized by autoradiography.

Paragraph beginning at line 23 of page 53 has been amended as follows:

For pre-screening of differential display cDNA clones, PCR-amplified cDNAs from different mRNA populations were generated following the procedures of Kelly *et al.* (1990), with minor modifications. Suspensor (6 DAP), ovule, 2 DAP seed, 3

DAP seed, 6 DAP micropylar region, 6 DAP chalazal region, and leaf total RNAs were isolated. First-strand cDNA was generated from 5 µg of each RNA using MMLV reverse transcriptase and 50 ng/µl of oligo(dT<sub>20</sub>) (SEQ ID NO:36) as primer. The first-strand cDNAs were 3' tailed with poly(dA) using terminal transferase. PCR amplifications were carried out using tailed first-strand cDNAs as templates and 2 µM of dT<sub>20</sub>dN (SEQ ID NO:37) (where dN = dG, dC, dA, or dT) as primer in 100 µl containing 20 mM Tris (pH 8.4), 50 mM KCl, 1 mM MgCl<sub>2</sub>, and 0.2 µM dNTPs at 94°C/1 minute, 42°C/2 minutes, and 72°C/5 minutes for 30 cycles, followed by a 10 minute extension at 72°C. A 1 µl aliquot from each reaction was used to perform another round of amplification using the same conditions. The reactions were extracted with phenol/chloroform and precipitated in ethanol. An aliquot equivalent to 1 µg from each reaction was size-fractionated in a 1% agarose gel, which was then used for DNA gel blot analysis according to the procedures of Sambrook *et al.*, *supra*.

Paragraph beginning at line 28 of page 55 has been amended as follows:

The region surrounding the ATG start codon in *G564g7.2.79* was converted into an SphI endonuclease restriction site by PCR using a T3 primer and a mutagenic oligo (5'-ATTGGACTGCATGCTTACGCTAGTCTGTGCAGAG-3'; SEQ ID NO:38). A 4.2 kb *G564* promoter region was cloned in the SphI site upstream of the *E coli β-Glucoronidase (GUS)* gene coding region (Jefferson, R. A., *et al.*, *EMBO. J.*, 6(13):3901-3907 (1987)) in pGEM5*GUS*. After cloning, the *G564* promoter region was re-sequenced. pGEM5*GUS* was constructed by inserting the *GUS* coding region and the Ti-plasmid *gene 7 3'* end from *TPI2/GUS* gene (Drews, G. N., *et al.*, *Plant Cell*, 4:1383-1404 (1992)) into the NcoI/NotI sites of pGEM5 (Promega: Madison, WI). The *G564/GUS* gene was transferred to the pHYGA (Hygromycin<sup>R</sup>) plant transformation vector (Klucher, K. M., *et al.*, *Plant Cell*, 8:137-153 (1996)). Tobacco plants were

transformed and regenerated using the leaf disk procedure of Horsch *et al.* (Horsch, *et al.*, *Science*, 227:1229-1231 (1985)).

Paragraph beginning at line 13 of page 63 has been amended as follows:

A comparison of the Scarlet Runner Bean G564 promoter (SEQ ID NO:1) and the Scarlet Runner Bean C541 promoter identified a conserved 10 base pair sequence which may confer suspensor-specific activity. Supporting this assertion, the sequence, GAAAAGCGAA (SEQ ID NO:10), is found at positions -846 to -837, i.e., within the area which the deletion results indicate controls suspensor-specific activity. Identical motifs can also be found at positions -1144 through -1135 and between -713 through -704 of Figure 1. The motif is also found at positions -684 through -675 of the Scarlet Runner Bean C541 promoter region (Figure 4). Interestingly, the Arabidopsis G564 ortholog promoter region comprises a motif (GAAAAGCCAA - ~~SEQ ID NO:12~~ ~~SEQ ID NO:11~~) that is highly homologous to SEQ ID NO:10.